541

An Unbiased Screen Identifies a CD137×PD-L1 Bispecific IgG1 Antibody With Unique T-Cell Activation and Binding Properties

Presented at the
AACR Annual Meeting 2019
Atlanta, GA, USA March 29-April 3, 2019

Cecile Geuijen,[1] Paul Tacken,[1] Rinse Klooster,[1] Horacio Nastri,[2] Shaun Stewart,[2] Jing Zhou,[2] Steve Wang,[2] Cheng-Yen Huang,[2] Arjen Kramer,[1] Linda Kaldenberg-Hendriks,[1] John de Kruif,[1] Renate den Blanken-Smit,[1] Vanessa Zondag-van der Zande,[1] Abdul Basmeleh,[1] Willem Bartelink,[1] Patrick Mayes,[2] Gregory Hollis,[2] Reid Huber,[2] Mark Throsby[1]

[1]Merus NV, Utrecht, The Netherlands [2]Incyte Corporation, Wilmington, DE

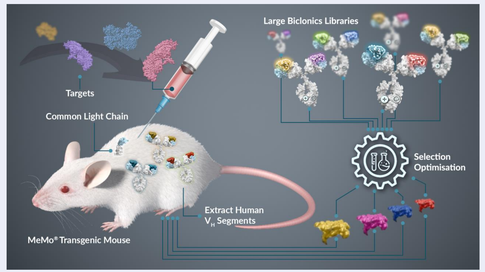
Abstract

CD137 (4-1BB) is a transmembrane costimulatory receptor on T and NK cells that enhances adaptive immune responses and is a critical mediator of antitumor immunity. CD137 signaling requires receptor clustering normally facilitated by the trimeric CD137 ligand (CD137L). Alternatively, CD137 signaling can be triggered either directly by agonistic monoclonal antibodies (mAbs) or indirectly via crosslinking of CD137 binding mAbs by Fcγ receptors on neighboring cells. The development of CD137 targeted agents for cancer therapy has been hampered by on-target off-tumor toxicity in the case of agonist, monospecific, bivalent mAbs or limited antitumor activity in the case of crosslinking mAbs. To address the issues of toxicity and efficacy, a highly selective and potent CD137xPD-L1 bispecific antibody (bAb) was identified by applying an unbiased functional screening approach. Collections of common light chain Fabs recognizing CD137 and PD-L1 were produced based on antibody panels from immunized MeMo® mice. A large and diverse panel of CD137xPD-L1 bAbs was then produced by combining different CD137 and PD-L1 Fabs based on epitope and sequence diversity in the IgG1 Biclonics® format. The bAbs were screened for activity in reporter cell lines expressing the receptors. This unbiased combinatorial screening identified a CD137xPD-L1 bAb (MCLA-145) for which CD137-mediated activation is dependent on the presence of PD-L1 on a neighboring cell and, as such, the antibody acts in 'trans'. Flow cytometry experiments demonstrated that MCLA-145 is fully cross-reactive to cynomolgus monkey CD137 and PD-L1. The CD137 Fab arm blocks the interaction of CD137 with CD137L as demonstrated in a competition assay by flow cytometry. The PD-L1 Fab arm blocks the interaction between PD-1 and PD-L1 as demonstrated in ELISA. Binding epitopes were mapped by shotgun mutagenesis using a flow-based screen. In addition, hydrogen-deuterium exchange experiments were performed to map the binding domain on CD137. Data show that MCLA-145 binds the ligand binding domain of CD137 domain (CRDII). The PD-L1 Fab arm binds PD-L1 in the PD-1 binding N-terminal V domain. Both epitope mapping data sets are consistent with the CD137 and PD-L1 ligand blocking activity of MCLA-145. Monovalent binding affinities were measured by surface plasmon resonance (SPR) and radioactive iodine labeling and demonstrated affinities in the low nM (CD137) and subnanomolar (PD-L1) range. SPR experiments also confirmed that MCLA-145 was able to bind simultaneously to both CD137 and PD-L1 recombinant proteins. The unique binding properties of MCLA-145 may result in an increased therapeutic window by specifically activating CD137 expressing cells in the tumor niche where PD-L1 is expressed while simultaneously blocking inhibitory input from the PD-1/PD-L1 axis.

Introduction

- CD137 (4-1BB) is a transmembrane costimulatory receptor on T and NK cells that enhances adaptive immune responses and is a critical mediator of antitumor immunity
- The development of CD137-targeted agents for cancer therapy has been hampered by on-target off-tumor toxicity
- CD137 signaling requires receptor clustering by the trimeric CD137 ligand, agonistic monoclonal antibodies (mAbs), or indirectly via cross-linking of CD137-binding antibodies by Fcγ receptors on neighboring cells
- PD-L1 expression is frequently observed on tumor cells and mAb-based PD-L1 inhibitors have demonstrated durable tumor remission in patients with diverse advanced cancers in the clinic
- We generated a diverse panel of CD137×PD-L1 bispecific antibodies (Biclonics®) to identify Biclonics® that would only activate CD137 in the presence of PD-L1

Antibody Generation

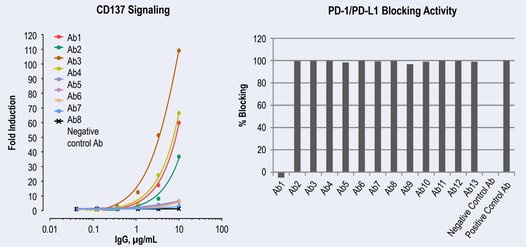


V_H, heavy chain variable domain

- The MeMo® and Biclonics® technoloies were used to generate MCLA-145
  - MeMo® is a transgenic mouse that generates human common light chain (cLC) antibodies
  - Biclonics® are bispecific human IgG1 molecules comprising 2 different cLC and heavy chain variable region binding domains, and a Fc region that preferentially forms the bispecific heterodimer via charge engineering
- To discover MCLA-145, a large diverse library of Biclonics® was generated
  - MeMo® mice were immunized with protein or DNA encoding CD137 or PD-L1
  - High-affinity, target-specific human Fab fragments were selected via phage display technology based on the repertoire generated in MeMo®
  - Selected cLC Fabs were reformatted as Biclonics® to generate libraries
  - The CH2 region of these Biclonics® was engineered to block Fcγ receptor interaction

Screening CD137 and PD-L1 Panels

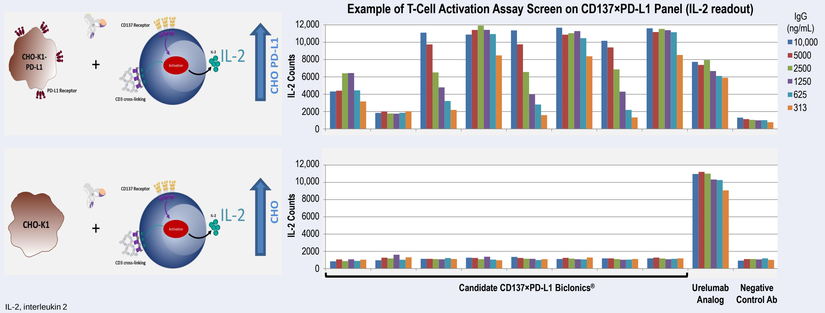
- Large cLC mAb panels were generated for CD137 and PD-L1, and Fab clones were selected for screening based on V_H sequence diversity
- Panels were screened for affinity, cross-reactivity with cynomolgus monkey and mouse orthologues, PD-1 and CD137 ligand (CD137L) blocking, domain-binding specificity, and ability to induce CD137 receptor activation



Representative samples of the bivalent cLC Ab screening. CD137 panel was screened using a CD137 expressing Jurkat reporter cell line (left). Displacement of PD-L1 by anti-PD-L1 Abs in ELISA format (right).
Ab, antibody

Unbiased Screening CD137×PD-L1 Biclonics® Library

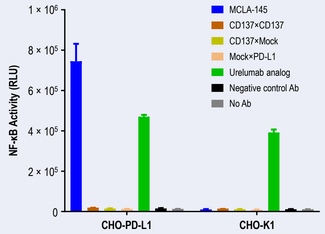
- A library of ~200 CD137xPD-L1 Biclonics® was produced and purified
- CD137xPD-L1 Biclonics® were screened in reporter and/or T cell transactivation assay in the absence and presence of PD-L1 expressing cells
- CD137xPD-L1 Biclonics® potentially activate T cells in the presence of PD-L1



IL-2, interleukin 2

MCLA-145–Mediated CD137 Activation Depends on Activation in 'trans'

- MCLA-145 induces CD137 signaling in CD137 Jurkat NF-kB/luc reporter cells in the presence of PD-L1 expressing CHO-PD-L1 cells, but not control cells without PD-L1 (CHO-K1)
- Positive control reference bivalent anti-CD137 urelumab analog induces CD137 signaling regardless of PD-L1 expression
- Bivalent monospecific mAb carrying 2 MCLA-145 CD137 Fab arms fails to induce CD137 signaling
- No CD137 signaling with control Abs carrying the MCLA-145 CD137 or PD-L1 Fab arm combined with mock arms (CD137×Mock and Mock×PD-L1)



RLU, relative light units

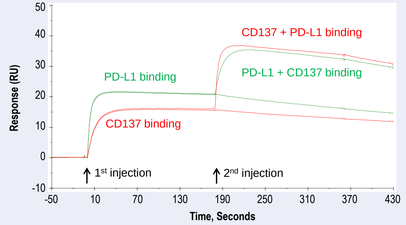
MCLA-145 Target Binding Characteristics

- The strength of MCLA-145 for CD137 and PD-L1 was assessed using radiolabeling and cell-based assays as well as surface plasmon resonance (SPR)

Antibody	Human (SPR)	Cynomolgus (SPR)	Human ^{125}I on Cells
K_D CD137	1.9 nM	0.6 nM	2.02 nM
K_D PD-L1	0.51 nM	0.32 nM	0.49 nM

K_D, dissociation constant

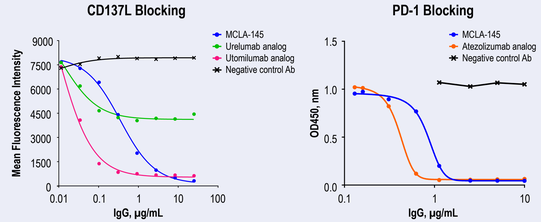
- MCLA-145 binds human PD-L1 while already in complex with CD137 as well as CD137 while already in complex with PD-L1



RU, response units.

MCLA-145 Blocks Ligand Binding

- Binding of CD137L to CD137 is blocked by MCLA-145 in a FACS-based ligand binding assay
- MCLA-145 blocks PD-1 binding to PD-L1 in an ELISA-based assay
- Analogs are bivalent antibodies



MCLA-145 Epitope Binding to CD137

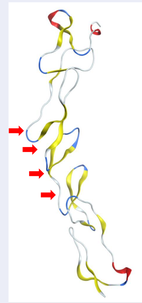
- Binding epitopes were identified using CD137 alanine shotgun mutagenesis libraries and Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS)
- MCLA-145 binds the ligand binding domain, CRDII/2, of CD137

CD137 Mutations Affecting MCLA-145 Binding

CD137 binding reactivity (%WT)

Mutation	MCLA-145	Control Ab
R66A	4.54	73.4
G70A	-1.7	70.6
V71A	13.2	93.0
F72A	-2.7	74.4

WT, wild type



Conclusions

- **MCLA-145 is an Fc-silenced Biclonics® that engages human CD137 and PD-L1 and blocks ligand binding to both receptors**
- **MCLA-145 induces CD137 signaling provided PD-L1 is present in its environment**
- **The unique binding properties of MCLA-145 may result in an increased therapeutic window by specifically activating CD137-expressing cells in the tumor niche where PD-L1 is expressed, while simultaneously blocking inhibitory input from the PD-1/PD-L1 axis**

Disclosures

Horacio Nastri, Shaun Stewart, Jing Zhou, Steve Wang, Cheng-Yen Huang, Patrick Mayes, Gregory Hollis, Reid Huber: Employment and stock ownership – *Incyte Corporation.* **Cecile Geuijen, Paul Tacken, Rinse Klooster, Arjen Kramer, Linda Kaldenberg-Hendriks, John de Kruif, Renate den Blanken-Smit, Vanessa Zondag-van der Zande, Abdul Basmeleh, Willem Bartelink, Mark Throsby:** Employment and stock ownership – *Merus NV*

Acknowledgments

This study was sponsored by Merus NV (Utrecht, The Netherlands) and Incyte Corporation (Wilmington, DE). Editorial, graphics, and printing support was provided by Envision Pharma Group, Inc. (Philadelphia, PA), funded by Incyte Corporation.

Scan code to download a copy of this poster